REMARKS

In the Office Action mailed May 20, 2002, the Examiner denied Applicant's request for amending the specification in view of the substitute specification submitted on March 4, 2002 because the specific entries on pages 2-8 of the amendment filed on March 4, 2002 could not be found in the substitute specification. Further, the Examiner objected to the substitute specification because of a number of informalities in the substitute specification. The amendments to the specification submitted in this Response are directed to the original specification as filed. A corrected substitute specification that overcomes the Examiner's objections in the present Office Action is also enclosed with this Response. The amendments to the specification are entirely cosmetic and/or incorporate proper headings, etc. in accordance with 37 CFR 1.77(b). The undersigned, a registered patent attorney, asserts that the substitute specification contains no new matter.

Each replacement is delineated by an instruction directed to a page and line and a request to insert the rewritten paragraph or section, or to replace the term. A clean version of the replacement paragraphs/sections is again submitted in this response in accordance with 37 CFR 1.121(b)(1)(ii) on pages 1-13.

Upon entry of the present amendments, claims 1-5, 11 and 14-20 are pending.

Claims 1, 11 and 14 have been amended.

New claims 15-20 are submitted. Support for the newly submitted claims may be found in the originally filed specification on pages 3, 5 and throughout the detailed description.

The amendments and newly submitted claims add no new matter.

Drawings

Figures 1-29 as originally recited in the specification were enclosed with the Amendment submitted on November 19, 2001. Applicant's note that the figures were originally part of the international application which was transmitted by the International Bureau. Applicants have taken note of the objections to the drawings and will provide formal figures upon receipt of a Notice of Allowance.

35 USC § 102

The Examiner has again rejected claims 1-4 under 35 U.S.C. § 102(b) as allegedly anticipated by Boullerne *et al.*, J. of Neuroimmunology 60: 117-124 (1995) ("*Boullerne*"). The Examiner states that Applicant's arguments filed on 3/4/02 have been fully considered but are not found persuasive. The Examiner recites that in spite of the amendments submitted in the Response filed on 3/4/02, claim 1 still reads on the reference antibody. The Examiner states that a product is a product regardless of how it is made and regardless of how it is used.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. MPEP § 2131. "There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention." *Scripps Clinic & Research Foundation v. Genentech Inc.*, 18 USPQ 2d 1001, 1010 (Fed. Cir. 1991). Here, *Boullerne* does not disclose the claimed invention.

Boullerne does not anticipate amended claims 1-4. As amended, claim 1 is directed to a purified antibody wherein the antibody **recognizes and binds specifically** to a nitrosylated protein **such that said purified antibody neutralizes the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates in a subject**. *Boullerne* concerns the nitrosation of amino acid conjugates and the use of NO-amino acid-g-BSA conjugates to coat a plate for an enzyme-linked immunosorbent assay (ELISA) experiment testing multiple sclerosis sera. *Boullerne* does not provide any teaching directed to a purified antibody that recognizes and binds specifically to a nitrosylated protein such that the purified antibody neutralizes the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates. *Boullerne* recites only that the antibodies simply recognize nitrosylated proteins.

Accordingly, *Boullerne* does not recite each and every element of amended claim 1. Claims 2-4 depend directly from amended claim 1 and thus incorporate all the limitations of amended claim 1 therein. For the same reasons stated with regard to amended claim 1, claims 2-4 are not anticipated by *Boullerne*. Consequently, claims 1-4 are novel over *Boullerne*.

Applicants also submit new claims 15-20. Claims 15- 20are directed to a method of treating the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates in a subject by administering to said subject an purified antibody where the antibody recognizes and binds specifically to a nitrosylated protein.

Boullerne does not recite each and every element of the method recited new independent claim 17. Claims 18-21 depend directly from amended claim 17 and thus incorporate all the limitations of amended claim 17 therein. Applicants respectively submit that new claims 17-21 are allowable and a notice of allowance is earnestly solicited.

35 U.S.C. §103

The Examiner has rejected claims 1-3, 5 and 11 under 35 U.S.C. § 103(a) as being unpatentable over *Boullerne* or Stamler *et al.*, Proc. Natl. Acad. Sci. USA 89: 444-448 (1992) ("*Stamler*") each in view of U.S. Pat. No. 6,090,382 to Salfeld *et al.* ("The '382 patent") and Campbell *et al.*, Monoclonal Antibody Technology, Elsevier Science Publishers (1984)("*Campbell*") or Harlow *et al.*, in Antibodies a Laboratory Manual, 1988, Cold Spring Harbor Laboratory Publication ("*Harlow*").

It is well recognized under U.S. law, that any rejection of a claim for obviousness over a combination of prior art references must establish that: (1) the combination produces the claimed invention; and (2) the prior art contains a suggestion or motivation to combine the prior art references in such a way as to achieve the claimed invention. (*In re Vaeck*, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991). In addition, the Examiner's prima facie case must include a finding that one of ordinary skill in the art at the time the invention was made would have reasonably expected the claimed invention to work. (*See In re O'Farrell*, 7 U.S.P.Q.2d 1673, 1681 (Fed. Cir. 1988); *In re Dow Chem.*, 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

Stamler teaches that proteins can be S-nitrosylated and that S-nitrosylation of proteins endows these molecules with potent and long-lasting endothelium-derived relaxation factor (EDRF)-like effects of vasodilation and platelet inhibition that are mediated by guanylate cyclase activation. *Stamler* also teaches methods for preparing S-nitroso proteins.

The '382 patent is directed to human antibodies that specifically bind to human tumor necrosis factor α (hTNFα). The Office Action recites that the '382 patent teaches a pharmaceutical composition comprising an antibody that binds to human TNFα and a pharmaceutical acceptable carrier or excipient such as sterile saline (See Col. 20, lines 57, bridging Col. 21, lines 1-52, in particular).

Campbell is a generalized reference directed to a discussion of the general properties and applications of **monoclonal antibodies** compared with antiserum. With regard to the purity of mononclonal antibodies, *Campbell* discusses only that bovine immunoglobulin obtained from foetal calf serum may have contaminating proteins contained therein that may be removed by affinity chromatography.

According to the Office Action, *Harlow* teach a method of producing monoclonal antibodies (See page 139-149, in particular); Harlow further teach that the advantages of monoclonal antibodies are their specificity of binding, their homogeneity and their ability to be produced in unlimited quantities (See page 141, last full paragraph, in particular).

The Examiner states that it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to produce monoclonal antibody as taught by *Campbell* or *Harlow* with the ntirosylated protein as taught by *Boullerne* or *Stamler* for a pharmaceutical composition comprising a monoclonal antibody that binds specifically to the nitrosylated protein and a pharmaceutical excipient as taught by the '382 patent, *Campbell* and *Boullerne* or *Stamler*. From the combined teachings of the references, the Examiner states that it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

As recited above in response to the rejection under 35 U.S.C. § 102, *Boullerne* does not teach or suggest a purified antibody that recognizes and binds specifically to a nitrosylated protein such that said purified antibody neutralizes the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates in a subject. *Boullerne* teaches only that the antibodies recognize nitrosylated proteins. In this regard one skilled in the art would not be motivated by the combination of the teaching of *Boullerne* with the teaching of the '382 patent, *Campbell*, *Harlow* or *Stamler*, to prepare a pharmaceutical composition comprising a monoclonal antibody that recognizes and binds specifically to a nitrosylated protein and neutralizes properties of the nitrosylated protein it recognizes.

Even if the way to obtain S-nitrosoprotein is known as taught in *Stamler*, and even if techniques are well-known to produce antibodies, such as described *Campbell* and/or *Howell*, it cannot be said that one skilled in the art would deem it obvious from a combination of these references to produce purified antibodies which recognize and specifically bind to a nitrosylated

protein such that said purified antibody neutralizes the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates in a subject as recited in amended claim 1. One skilled in the art is faced with several obstacles to obtain such purified antibodies capable of recognizing *in vivo* S-nitrosoproteins, and which neutralize their properties. For example, to obtain anti-nitroso-cysteine antibodies as described in the present application, the Applicants had to conjugate the cysteine according to a process in which the cysteine is an immunogen and the NO is an immunodominant element of the antigen. Moreover, in the monoclonal approach as described on pages 37-43 of the specification of the instant application, to stimulate a sufficient population of lymphocytic clones for hybridization, particular methods of administration of the immunogen were used to obtain an immunogenic response directed to NO-cysteine. The lymphocytic hybridization and the monoclonal selection require knowledge and know-how not taught in any of the cited prior art references.

Thus, neither *Stamler* nor the '682 patent, alone or in combination with *Campbell* teach or suggest a **purified antibody** wherein the antibody recognizes and binds specifically to a nitrosylated protein such that said purified antibody neutralizes the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates in a subject. Furthermore, neither *Stamler* nor the '101 patent, alone or in combination with *Campbell* teach or suggest a purified polyclonal or monoclonal antibody. Based on the disclosures of *Stamler* and the '101 patent, one skilled in the art would not be motivated to combine either of these references with the teaching of *Campbell* to provide **a purified antibody wherein the antibody recognizes and binds specifically to a nitrosylated protein such that said purified antibody neutralizes the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates in a subject** as recited in amended claim 1. There is no explicit nor implicit suggestion or motivation to combine the teachings of *Boullerne* or *Stamler* with the teaching of the '682 patent, and *Campbell* or *Harlow* so as to make the precise choices necessary to arrive at the purified antibody of amended claim 1. Amended claim 1 is thus not obvious. Claims 2, 3 and 5 depend directly from claim 1 and incorporate all the limitations of amended claim 1 therein. As such, claims 2, 3 and 5 are likewise not obvious in view of the cited prior art for the same reasons as recited with regard to amended claim 1.

Claim 11 has been amended to recite a pharmaceutical composition comprising: (a) a purified antibody that recognizes and binds specifically to a nitrosylated protein; and (b) a pharmaceutically acceptable vehicle, wherein said purified antibody neutralizes the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates in a subject. While the '382 patent teaches a pharmaceutical composition, it is directed to a pharmaceutical composition comprising an antibody that binds to human TNFα and a pharmaceutical acceptable carrier or excipient such as sterile saline. There is no teaching or suggestion in the '382 patent, alone or in combination with *Stamler* or *Campbell* directed to the pharmaceutical composition recited in amended claim 11 for reducing the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates in a subject.

In view of the foregoing amendments and arguments, it is respectfully submitted claims 1-3, 5 and 11 are not obvious in view of the references cited by the Examiner, and that the present rejections under 35 U.S.C. §103(a) should be withdrawn

The Examiner has rejected claim 14 under 35 U.S.C. § 103(a) as being unpatentable over *Boullerne* or Stamler *et al.*, Proc. Natl. Acad. Sci. USA 89: 444-448 (1992) ("*Stamler*") each in view of U.S. Pat. No. 5,919,543 ("the '543 patent") and Campbell *et al.*, Monoclonal Antibody Technology, Elsevier Science Publishers (1984)("*Campbell*") or Harlow *et al.*, in Antibodies a Laboratory Manual, 1988, Cold Spring Harbor Laboratory Publication ("*Harlow*") as applied to claims 1-3, 5 and 11 above, and further in view of U.S. Pat. No. 5,858,682 ("the '682 patent") for the same reasons set forth in Paper No. 10.

In Paper No. 10, the Examiner recited that the claimed invention in claim 14 differs from the references only by the recitation of a kit comprising antibody for detection of any nitrosylated proteins in a biological specimen. The Examiner further stated in Paper No. 10 that the '682 patent teaches a kit comprising antibody for diagnostic (see col. 3, line 40; col. 6, line 17; col. 8, line 36, in particular.

The *Boullerne*, *Stamler*, *Campbell*, and *Harlow* references were described and discussed infra with regard to the rejection to claims 1-3, 5 and 11.

The '682 patent discloses a monoclonal antibody which binds specifically with an E2A/pbx1 fusion epitope. Contrary to the Examiner's assertion, the '682 patent discloses in col.

3, lines 46, that optimal panels of mAbs(E2A/pbx1 junction and pbx1) for each application will be selected. According to the '682 patent, these will serve as basis for a **clinical kit** development for childhood pre-B leukemia. Such a **diagnostic kit** will allow clinical reference laboratories to screen a heterogeneous population of cells from blood or bone marrow for the presence of the E2A/pbx1 fusion protein resulting in the fast and reliable detection of t(1;19) bearing leukemia cells. In col. 8, lines 36-41, the '682 patent recites that a "**diagnostic system in kit form** of the present invention includes, in an amount sufficient for at least one assay, a polypeptide, polypeptide admixture, antibody composition or monoclonal antibody composition of the present invention, as a packaged reagent. Instructions for use of the packaged reagent are also typically included." The diagnostic system of the '682 patent may further include a binding agent. The '682 patent teaches that the polypeptide, antibody molecule composition or monoclonal antibody molecule composition of the invention can be affixed to a solid matrix to form a solid support that comprises a package in the subject diagnostic systems.

With all due respect, Applicants note that the '543 recited in the rejection to claim 14 is entitled "Composite sine wave spar". Applicants do not believe this is the patent the Examiner wished to cite. Additionally, Applicant's note that the Examiner's reference to US Pat. No. 5,919,543 in the Office Action is followed by a parenthetical that states "(July 1999, PTO 892). Applicants note that this application was filed in November 1999. Since Applicants are not able to decipher the patent that the Examiner intended to cite, Applicants request withdrawal of the finality of the present Office Action in order to have the opportunity to review the patent intended to be cited by the Examiner.

Amended claim 14 recites a kit for *in vitro* detection of nitrosylated proteins in a biological specimen comprising (a) a purified antibody that recognizes and binds specifically to a ntirosylated protein and (b) reagents to produce a medium favorable for an immunological reaction between said antibody and any nitrosylated proteins that may be present in a biological specimen.

The Examiner asserts that it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the antibody in a kit taught by the '682 patent with the antibody that binds nitrosylated protein as taught by *Boullerne*, *Stamler*, *Campbell*, and *Harlow* for the detection of nitrosylated protein immune complex in any biological specimen as

taught by *Boullerne*. As recited above, the '682 teaches a "diagnostic system in kit form" for performing an assay. The '682 patent discloses that the diagnostic system is used for detecting acute lymphoblastic leukemia using the specific antibodies disclosed in the patent. The '682 patent also teaches that the ingredients of its diagnostic system are affixed on a solid support. The kit recited in amended claim 14 of the instant application is directed to the *in vitro* detection of nitrosylated proteins in a biological specimen. There is thus no teaching or suggestion in the '682 patent directed to a kit for *in vitro* detection of nitrosylated proteins in a biological specimen. Nor does the '682 patent teach or suggest a kit which comprises a purified antibody that recognizes and binds to specifically to a nitrosylated protein, and reagents to produce a medium favorable for an immunological reaction between the purified antibody and any nitrosylated proteins that may be present in a biological specimen. The '682 patent provides no teaching or suggestion directed to producing a medium favorable for an immunological reaction between the purified antibody and any nitrosylated proteins that may be present in a biological specimen in a kit as recited in amended claim 14. Accordingly, there is no motivation or teaching to suggest to one of skill in the art to combine the '682 patent with the teachings of *Boullerne* or *Stamler*, each in view of *Campbell* or *Harlow* as applied to claims 1-3, 5 and 11 above, and further in view of the '682 patent, in order to arrive at the kit as recited in amended claim 14.

In view of the foregoing amendments and arguments, it is respectfully submitted that the present claims are not obvious in view of the references cited by the Examiner. Applicants respectfully request reconsideration and withdrawal of the present rejection.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

CONCLUSION

On the basis of the foregoing amendments, the points and concerns raised by the Examiner having been addressed in full, Applicants respectfully submit that the pending claims are in condition for allowance, which action is respectfully requested.

If, upon receipt and review of this amendment, the Examiner believes that the present application is not in condition for allowance and that changes can be suggested which would place the claims in allowable form, the Examiner is respectfully requested to call Applicant's undersigned counsel at the number provided below. **Furthermore, if the Examiner is of the believe that the claims are not yet in condition for allowance, Applicants respectfully request the withdrawal of finality to continue prosecution of the claims.**

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

On page 1, line 4, please insert the following heading and paragraph:

--CLAIM OF PRIORITY

This application is a 35 U.S.C. 371 national application of PCT/FR97/02412, filed on December 23, 1997. The application also claims priority to French Patent Application FR96/16207.

--TECHNICAL FIELD OF THE INVENTION--.

On pg. 1, line 14, insert --BACKGROUND OF THE INVENTION--.

Please replace the paragraph beginning on page 1, line 15, with the following rewritten paragraph:

-- Nitric oxide, hereinafter designated also as NO, is described as being the smallest molecule made by the cells. Initially assimilated to endothelium derived relaxing factor (EDRF), it was then recognized as a neuromediator, and is thought to be the first neurotransmitter with retrograde activity, as well as a cytostatic/cytotoxic molecule. Because of its strong reactivity, nitric oxide is capable of reacting with a large number of molecules to form conjugates which have multiple functions and therefore participate in many physiological and pathophysiological processes.--

On page 2, line 27, insert --SUMMARY OF THE INVENTION--.

On page 2, line 28, insert --DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph illustrating the zone of absorbency of NO-Tyr-BSA.

Fig 2 is a graph illustrating the zone of absorbency of NO-Cys-BSA.

Fig. 3 is a graph illustrating response following immunization with NO-Tyr-BSA.

Fig. 4 is a graph illustrating response following immunization with NO-Cys-BSA.

Fig. 5 is a graph illustrating the avidity of conjugated anti-NO-Tyr Ab and conjugated anti-NO=Cys in competition tests.

Fig. 6 illustrates the kinetics of the formation and concentration in NO-Cys-BSA formed in supernatant of the culture of activated macrophages determined at incubation times: 0, 3, 4, 6, 8, 11, 14, 18 and 20 hours using "C" antiserum.

Fig. 7 illustrates inhibition of the cytostatic effect of the BCG macrophages on the *T. musculi in vitro* in the presence of NMMA (0.5 mM), ("T") or ("C") antiserum used at 1/100.

Fig. 8 illustrates the cytostatic effect of supernatants containing NO-BSA from activated macrophages added to normal macrophages containing *T. musculi*. Inhibition of this effect in the presence of NMMA (0.5 mM), ("T") or ("C") antiserum used at 1/100.

Fig. 9 illustrates the spectrometric analysis of the NO-Cys-G-BSA immunogen and its structural homologue Cys-G-BSA based on wavelength.

Fig. 10 illustrates the evolution of the antibody response during immunization of the intraperitoneally immunized mouse.

Fig. 11 illustrates the evolution of the antibody response during immunization of the intraperitoneally immunized mouse.

Fig. 12 illustrates the avidity and specificity of the anti-NO-Cys-G antibodies in the mouse (IP).

Fig. 13 illustrates represents the avidity and specificity of the anti-NO-Cys-G mouse antibodies (PC).

Fig. 14 illustrates the avidity and specificity of the anti-NO-Cys-G monoclonal Ab.

Fig. 15a is a high-magnification (100X) immunocytochemical marking illustrating anti-NO-Cys-G monoclonal antibody and showing very clear markings (immunoreactivities) in terms of trypanosomes co-cultivated in the presence of the activated macrophages.

Fig. 15b is a high-magnification (100X) immunocytochemical marking illustrating a much weaker marking obtained in the co-culture of activated macrophages / trypanosomes, in the presence of NMMA (0.5 mM).

Fig. 15c is s a high-magnification (100X) immunocytochemical marking illustrating a total absence of trypanosome marking was obtained when a normal mouse serum was used.

Fig. 15d is a high-magnification (100X) immunocytochemical marking illustrating antibody "C" having an intensity very close to the one for the monoclonal Ab.

Fig. 15e is a high-magnification (100X) immunocytochemical marking illustrating the anti-NO-Tyr ("T") giving a positive marking with an intensity not as high as the two types of antibodies (monoclonal and polyclonal) directed against the epitope NO-Cys.

Fig. 15f is a high-magnification (100X) immunocytochemical marking illustrating the absence of marking in the primary Ab of a normal rabbit.

Fig. 16 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-BSA epitope.

Fig. 17 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on delipidated NO-BSA epitope.

Fig. 18 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Cys-BSA epitope.

Fig. 19 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Cys-G-BSA epitope.

Fig. 20 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Tyr-BSA epitope.

Fig. 21 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Tyr-G-BSA epitope.

Fig. 22 illustrates the OD values for the normal mouse serum and the parasitized mouse serum showing the responses on NO-Tryp-G-BSA epitope.

Fig. 23 illustrates the OD obtained from ELISA tests on the NO-Cys-G-BSA, NO-Tyr-BSA and NO_2-Tyr-BSA conjugates (the control group) and indicating presence of circulating Ab whose rate increases during attacks an ddecreases during remissions.

Fig. 24 illustrates the OD obtained from ELISA tests on anti-NO-Tyr-BSA and anti-NO2-Tyr-BSA conjugates (the aminoguanidine group).

Fig. 25 illustrates the OD obtained from ELISA tests on anti-NO-Cys-G, anti-NO-Tyr and anti-NO_2-Tyr and showing the changes over time in the anti-NO-Cys-G, anti-NO-Tyr and anti-NO_2-Tyr responses (the monoclonal antibody group);

Fig. 26 illustrates the progression of antibodies directed against NO-Cys-G-BSA, NO-Tyr-BSA and NO2-Tyr-BSA conjugates (the control group).

Fig. 27 illustrates the progression of antibodies directed against NO-Cys-G-BSA, NO-Tyr-BSA and NO2-Tyr-BSA conjugates (the NIS group).

Fig. 28 illustrates the progression of antibodies directed against anti-NO-Tyr-BSA and anti-NO2-Tyr-BSA conjugates (the aminoguanidine group); and

Fig. 29 illustrates the progression of antibodies directed against anti-NO-Cys-G, anti-NO-Tyr and anti-NO_2-Tyr conjugates (the monoclonal antibody group).

DETAILED DESCRIPTION OF THE INVENTION--.

On page 5, line 10, after the term "rejection,", please insert the word --and--; and after the term "neurotoxicity", please delete "..." and insert -.-.

On page 5, line 28, after the term "etc", please delete "..." and insert -.-.

Page 7, line 8, please delete "No-synthase", and insert --NO-synthase--.

Please replace the paragraph beginning at page 7, line 25, with the following rewritten paragraph:

--Several types of NOS were cloned and classified in two distinct families: NOS termed constitutive (cNOS) or [inducible] <u>inducible</u> NOS (iNOS)--.

On page 11, line 12, please delete "glutathion,...) and insert --and glutathione)--.

On page 11, line 27, please replace "-Reaction with tyrosine:", with -- -<u>Reaction with tyrosine</u>:--

On page 15, line 34, after the word "by", please delete the term "anti-IFNg" and insert --IFNγ--.

On page 16, line 1, after the term "factor", please delete the term "(TFN-a)" and insert the term --(TFN–α)--.

On page 16, line 30, after the term "dioxide", please delete the term "$N0_2$" and insert --NO_2--.

On page 16, line 34, after the term "compounds", please delete "(S-nitrocystein, S-nitrosoglutathion)" and insert --(S-nitrocysteine, S-nitrosoglutathione)

On page 17, line 9, after the word "or", please delete the term "anti-IFNg" and insert --IFNγ--.

On page 20, line 32, after the word "as", please delete the phrase "IFNg and TFN-a/b" and insert -- IFNγ and TFN-α/β--.

On page 21, line 13, please delete "(Ac)" and insert --(Ab)--.

On page 21, line 14, after "acids", please delete "(cystein," and insert --(cysteine,--.

On page 21, line 15, after the term "tyrosine", please delete the term "tryptophane..." and insert -- and tryptophan--; and after "tryptophane", please delete "..." and insert -.-.

On page 21, line 5, please delete "NO-cystein", and insert --NO-cysteine--.

On page 21, line 9, please delete "cystein-N-acetylated", and insert -- cysteine-N-acetylated--.

On page 21, line 11, please delete "conjugated", and insert --conjugate--.

On page 22, line 5, please delete "NO-cystein", and insert --NO-cysteine--.

Please replace the paragraph beginning at page 22, line 26, with the following rewritten paragraph:

-- Concentration (M) in coupled hapten = X mg hap x CPM [après [after]] after / CPM [avant [before]] before x Vol before x PM hap

where X mg is the quantity of hapten used for the coupling; CPM before is the radioactivity before dialysis; CPM [après] after is the radioactivity after dialysis; Vol before is the volume before dialysis; PM hap is the molecular weight of the hapten.--

Please replace the paragraph beginning at page 23, line 7, with the following rewritten paragraph:

--[Le] The coupling relationship is the number of moles of hapten coupled with a mole of protein:--

Please replace the sentence at page 23, line 11, with the following rewritten sentence:

-- The conjugated Weight = [(R x PMhap[}]) + PM prot] x conc protein.--

Please replace the paragraph beginning at page 25, line 9, with the following rewritten paragraph:

-- The nitrosylation method of two types of coupling (G [et] and SA) is identical to the one described above for the carbodiimide type of coupling.--

Please replace the paragraph beginning at page 25, line 11, with the following rewritten paragraph:

-- Synthesis of NO_2-tyrosine-BSA : The synthesis of this conjugate requires 20 mg of the NO_2-Tyr (Sigma) hapten and 20 mg of BSA. [Le] The coupling takes place with carbodiimide following the same protocol described above.--

Please replace the paragraph beginning at page 26, line 1, with the following rewritten paragraph:

-- The polyclonal serums were adsorbed on the corresponding non-nitrolysated conjugates: Tyr-BSA/HSA for the "T" rabbit and Cys-BSA/HSA for the "C" rabbit. (Geffard et

al., 1984a; Geffard et al., 1985b; Campistron et al., 1986). The adsorption took place in proportions of 5 mg of conjugate per ml of pure serum. The mixture was incubated for 16 hours at 4°C under agitation and the immunoprecipitates were eliminated by centrifugation for 15 minutes at [10 OOOg] 10000g. The supernatant is enriched in specific Ig, while the pellet contains the rabbit Ig-carrier protein immune complexes.--

Please replace the paragraph beginning at page 26, line 10, with the following rewritten paragraph:

-- To one volume of rabbit polyclonal serum an equal volume of a saturated ammonium sulfate solution $(NH_4)_2SO_4$ is added. The mixture was incubated for 1 hour at 4°C, and then centrifuged for 15 minutes at [10 OOOg] 10000g. The cell (containing the Ig precipitates) is taken up in a minimum volume of TPB buffer and then dialyzed for 3 days in a SPB buffer (Na_2HPO_4, 0.01 M, NaCl 0.15 M).--

Please replace the paragraph beginning at page 29, line 17, with the following rewritten paragraph:

-- Decrease in the OD (B) indicates the presence of competition between the conjugated hapten which is adsorbed on the microtitration plate and the hapten preincubated with the corresponding antiserum. Bo is the OD corresponding to the response obtained with the antiserum in the absence of the competitor. A dilution of the antiserum (1/20,000) yielding a OD of approximately 1.0 [à] to 492 nm was chosen for adjustment of the value of Bo; the B/Bo relationship was used to trace the competition curves of figure 5 obtained with the competitors.--

On page 29, line 17, after the term "1.0", please delete "à", and insert --to--.

On page 29, line 25, please delete "et", and insert --and--.

On page 31, line 13, delete the term "NEUTRALISATION", and insert --NEUTRALIZATION--.

On page 31, line 34, after the term "secrete", please delete "IFN-g", and insert --IFN-γ--.

On page 32, line 2, after the word "The", please delete "IFN-g", and insert --IFN-γ--.

On page 32, line 3, after the word "as", please delete the term "TNF-a", and insert --TFN-α--; and in the same line please delete "IFN-g", and insert --IFN-γ--.

On page 36, line 2, please delete "et", and insert --and--.

On page 41, line 25, after the word "to", please delete "4 x 10-9M." and insert -- 4 x 10^{-9} M.--

On page 42, line 27, after the word "to", please delete "4 x 10-9M." and insert -- 4 x 10^{-9} M.--

On page 43, line 10, after "0.085", please delete "et", and insert --and--.

On page 49, line 22, please replace "-NO-Cys-BSA et NO-Cys-G-BSA:" with -- -NO-Cys-BSA and NO-Cys-G-BSA: --

On page 51, line 25, please replace "-Direct cytotoxicity by NO.", with -- -Direct cytotoxicity by NO.--

On page 52, line 18, after the amino acid sequence, please insert --(SEQ ID NO.:1).--

Please replace the paragraph beginning at page 54, line 3, with the following rewritten paragraph:

-- -Recent work has shown the formation of nitrotyrosines at inflammatory sites (Kaur and Halliwell, 1994). To detect the presence of immunological responses to these epitopes in the serums of rats, [NO_2-Tyr-BSA] NO_2-Tyr-BSA and the conjugated nitrosotyrorine (NO-Tyr-BSA) were used. Tyr-BSA was used to correct OD obtained on: [NO_2-Tyr-BSA] NO_2-Tyr-BSA and NO-Tyr-BSA.--.

Please replace the paragraph beginning at page 60, line 16, with the following rewritten paragraph:

--The serums of the ten rats drawn for 5 weeks were tested on conjugates NO-Cys-G-BSA; NO-Tyr-BSA; [N02-Tyr-BSA] NO2-Tyr-BSA, and on the corresponding non-nitrosylated conjugates. Figures 26 and 27 show the "Control" and "NIS" groups, respectively, with the progression over time of the antibodies induced against two epitopes: [N02-Tyr-BSA] NO2-Tyr-BSA and NO-Tyr-BSA. These results represent the average on two tests. The OD obtained in each group are equivalent on all conjugates tested, each point represents the average and the standard deviation of the OD obtained with the 5 rats in the same group for the same conjugate.--

Please replace the paragraph beginning at page 61, line 1, with the following rewritten paragraph:

-- For "Aminoguanidine": (Figure 28) anti-NO-Tyr is the highest response; the OD are between 1.5 and 2 (between the 2nd and 5th weeks). The [anti-N02-Tyr] anti-NO2-Tyr response is as large; note an increase in signals between the 1st and 2nd weeks. They stabilize until the 3rd week, then increase slightly toward the 4th week.--

On page 63, line 3, please delete the term "IFN-g", and insert --IFN-γ--.

On page 63, line 17, please delete the term "N02-", and inert --NO_2^---.

On page 63, line 18, please delete "IFN^y/TNFa" and insert --IFN-γ/TNF-α--.

On page 63, line 19, please delete "anti-TNFa", and insert --anti-TNF-α--.

On page 63, line 32, please delete "IFN-g, TNF-a", and insert --IFN-γ, TNF-α--.

On page 65, line 31, please delete "NOBSA", and insert --NO-BSA--.

On page 70, line 21, please delete "NO or $N0_2$ NO-Cys-G, NO-Tyr and $N0_2$-Tyr.", and insert -- NO or NO_2, NO-Cys-G, NO-Tyr and NO_2-Tyr.--

Please insert a "-" before the first letter of the underlined subheading on the following pages/lines: page, 10, line 5; page 13, line 4; and page 13, line 21.

Please delete the "." in front of the first letter of the subheading on the following pages/lines and replace each occurrence with a "-": page 22, line 14; page 22, line 32; page 23, line 7; page 23, line 10; page 24, line 21; page 25, line 11; page 25, line 34; page 26, line 9; page 26, line 15; page 29, line 32; page 30, line 4; page 31, line 5; page 33, line 18; page 33, line 27; page 34, line 8; page 41, line 8; page 41, line 18; page 46, line 14; page 46, line 16; page 46, line 18; page 46, line 27; page 46, line 29; page 46, line 32; page 53, line 12; page 53, line 18; page 53, line 31; page 54, line 3; page 54, line 14; page 54, line 21; page 54, line 26; page 55, line 22; page 55, line 30; page 56, line 3; page 56, line 23; page 56, line 28; page 56, line 30; page 58, line 13; page 58, line 15; page 58, line 19; page 58, line 21; page 59, line 12; page 59, line 14; page 59, line 16; page 59, line 17; page 59, line 28, page 59, line 29; page 59, line 30; page 59, line 32; page 60, line 15; and page 62, line 5.

In the claims:

On pg. 89, line 2, kindly insert --We claim--.

1. (Twice Amended) A purified antibody, wherein the antibody recognizes and binds specifically to a nitrosylated protein such that said purified antibody neutralizes the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates in a subject.

11. (Three times amended) A pharmaceutical composition comprising:

(a) a purified antibody that recognizes and binds specifically to a nitrosylated protein; and

(b) a pharmaceutically acceptable excipient;

wherein said purified antibody neutralizes the deleterious effects of excessive or inadequate production of nitric oxide or its conjugates in a subject.

14. (Three Times Amended) A kit for *in vitro* detection of nitrosylated proteins in biological specimen, comprising :

(a) a purified antibody that recognizes and binds specifically to a nitrosylated protein; and

(b) reagents to produce a medium favorable for an immunological reaction between said purified antibody and any nitrosylated proteins that may be present in a biological specimen.